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ORIGINAL RESEARCH

Cold Stress Increases Lipolysis, FFA R_a and TG/FFA Cycling in Humans

ANDRÉ L. VALLERAND, PH.D., JIRI ZAMECNIK, PH.D.,
PETER J. H. JONES, PH.D., AND IRA JACOBS, DR. MED. SC.

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Background: To characterize the important changes in the selection and mobilization of metabolic fuel during cold stress, six males rested for 3 h at 29°C and at 5°C dressed only in shorts while $^2H_5$ glycerol, 1-^{13}C palmitate and 6,6 $^2H_2$ glucose were continuously infused for 3 h in each condition to determine their rate of turnover (R_a). ***Methods:*** Metabolic rate (M) as well as rates of carbohydrate (CHO_{ox}) and lipid oxidation (FAT_{ox}) were assessed by indirect calorimetry whereas all isotopic enrichments were determined by mass spectrometry. ***Results:*** Cold exposure decreased rectal and mean skin temperatures and increased M, FAT_{ox} and CHO_{ox} compared with the same test at thermal neutrality ($p < 0.05$). As expected, cold increased plasma glucose R_a and plasma FFA R_a (from 4.58 ± 0.19 to 14.69 ± 1.07 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $p < 0.05$). However, in absolute terms, plasma FFA R_a in the cold remained more than twice greater than FAT_{ox} (FAT_{ox} only increased up to 6.9 ± 0.85 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), suggesting an enhanced non-oxidative disposal of fatty acids (i.e., TG/FFA cycling) to account for all FFA R_a. Indeed, cold increased extracellular TG/FFA recycling rate (2.23 ± 0.40 vs 7.77 ± 1.19 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $p < 0.05$) whereas intracellular cycling was unaffected. ***Conclusion:*** Even though lipolysis and FFA R_a are greatly increased by cold stress in humans, the present results demonstrate that only about half the rate of FFA R_a is ultimately oxidized, suggesting that under the present cold conditions: 1) non-oxidative FFA disposal or TG/FFA cycling is significantly enhanced; 2) white adipose tissue-derived fatty acids could easily account for most of FAT_{ox}. The results further emphasize the importance of the TG/FFA cycle in amplifying the ability of stored TG to react quickly to major changes in energy expenditure induced by a sustained cold stress.

Keywords: body temperatures, energy metabolism, energy substrates, glycerol, heat balance, heat loss, heat production, non-oxidative disposal, shivering, stable isotope.

UNDER CIRCUMSTANCES of natural or man-made disasters, in military environments or under other conditions associated with either severe or prolonged cold exposure, the avoidance of hypothermia depends on balancing heat loss with heat production. In prolonged uncompensable cold stress where heat loss continuously adds to the heat debt, the first line of defense for survival, is to maximize the insulation provided by the microclimate and by peripheral vasoconstriction. The last line of defense for survival remains the cold-induced increase in metabolic heat production (4). The mobilization and the oxidation of metabolizable energy substrates provides the body with fuel for either work or heat (10).

Carbohydrates (CHO) represent an important source of energy for thermoregulatory thermogenesis. It has been demonstrated that cold exposure greatly enhances plasma glucose tolerance and turnover, as well as peripheral tissue glucose uptake and oxidation (10,16,26,27). Further, adipose tissue triglycerides (TG) represent the body's largest fuel reserve. Mobilization of this energy store is important during shivering to provide fuel for the contracting muscles. As such, several studies have shown that cold exposure in animals enhances plasma levels of free fatty acids (FFA), glycerol and catecholamines as well as lipolysis and FFA turnover (10,16,19). None of the previous studies, however, has compared in humans glycerol R_a (an index of whole body lipolysis) to FFA R_a and fatty acid oxidation in the cold to further characterize lipid metabolism and in particular to dissociate oxidative vs. non-oxidative FFA disposal. There are several examples where the availability of fatty acids exceeds the oxidation by an amount that corresponds to non-oxidative disposal (12,21,36). Whether the same phenomenon applies to cold stress is unknown.

The goal of this study was, therefore, to characterize the important changes in the metabolic fuel mobilization and selection during cold stress, with particular emphasis on oxidative vs. non-oxidative disposal of FFA. To accomplish these objectives, we combined the use of several techniques, namely the use of indirect calorimetry-derived rates of substrate oxidation and stable isotope tracer techniques where continuous infusions of $^2H_5$ glycerol, 1-^{13}C palmitate and 6,6 $^2H_2$ glucose were performed for 3 h, respectively, to determine the rate of lipolysis as well as the rate of appearance (R_a) or turnover of plasma FFA and glucose, both at thermal

From the Defence and Civil Institute of Environmental Medicine, Human Protection and Performance Sector (A. L. Vallerand and I. Jacobs) and Military Operational Medicine Sector, (J. Zamecnik), North York, Canada; and McGill University, School Dietetics and Nutrition, Montreal, Canada (P. J. H. Jones).

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Address reprint requests to: Dr. A.L. Vallerand, Deputy Scientific Advisor - Human Performance, Defence Research & Development Branch, National Defence Headquarters, 190 O'Connor, Ottawa ON K1A OK2 Canada.

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neutrality and in the cold. It is hypothesized that cold exposure enhances FFA R_a to a level that exceeds whole body lipid oxidation, in part due to nonoxidative disposal of fatty acids (or TG/FFA cycling).

METHODS

Subjects

The experimental protocol was approved by our institutional Human Ethics Committee. A physician examined all subjects and approved their participation. The nature, purpose and possible risks of the study were explained to each individual before they gave their written consent to participate. Prior to any tests, subjects were familiarized with the protocol during a 1.5-h familiarization trial. Six healthy males (Caucasian) volunteered for the present study. Maximal aerobic power ($\dot{V}O_{2max}$) was determined on the treadmill using an incremental protocol to exhaustion (32). Body fat was assessed via underwater weighing, as previously described (29). Two tests were performed about 1–2 wk apart in each subject who served as his own control (repeated measures design). The order of treatments was balanced. Subjects were asked to avoid alcohol for at least 48 h prior to the test, to avoid exercise for 24 h before, and to report to the laboratory in a 12–14 h postabsorptive state. Their mean (±SEM) physical characteristics were as follows: 30 ± 2 yr, 1.74 ± 0.02 m, 76.4 ± 3.1 kg, 49 ± 2 ml · kg^{-1} · min^{-1} $\dot{V}O_{2max}$, 14 ± 1% body fat and 1.911 ± 0.044 m^2 of body surface area.

Measurements

Early in the morning, subjects were instrumented with a rectal probe (American Pharmaseal Co., Valencia, CA), 12 re-calibrated heat flux transducers (Concept Engineering, Old Saybrook, NJ), a heart rate monitor (Polar Vantage XL, Polar USA, Stanford, CT) and 2 indwelling intravenous catheters. Then, they rested in a quiet room set at about 22°C for approximately 0.5 h before the test while sitting on the experimental chair (a nylon-webbed lawn chair which had been modified to wheel the subjects in and out of the chamber). The cold test consisted of exposing for 3 h at 5.0 ± 0.1°C air (semi-nude) subjects who were wearing only jogging shorts and thin foam slippers (negligible wind speed). The thermal neutrality test was performed in an identical manner to the above, but at 29.3 ± 0.1°C. During the tests, T_{re} was monitored using a thin thermistor probe inserted and secured 10 cm beyond the anus. Skin temperatures and dry heat losses were measured with 12 re-calibrated heat flux transducers taped to the skin. Using a 12 point area-weighted system, mean skin temperature (T_{sk}) and mean dry heat loss were calculated as described elsewhere (30). All thermal data were continuously recorded with a computerized data acquisition system (30).

O_2 consumption and CO_2 production (in L · min^{-1} STPD) were measured from expired air using a metabolic cart consisting of an IBM PC, a polarographic O_2 analyzer and an infrared CO_2 analyzer (Applied Electrochemistry, Pittsburgh, PA), a ventilation module (Interface Associates, Irvine, CA) and a Turbofit A/D computer interface (Vacumetrics, Ventura, CA). Respiratory gas exchanges were continuously measured for the entire 3-h period, with the exception of about 5 min beginning at min 85, where subjects were able to remove the metabolic face mask (Hans Rudolf, Kansas City MO) to avoid discomfort. Analyzers were recalibrated at that point in time (if required) and before any test. Urine collection took place after the tests to determine urinary urea nitrogen, used as an index of protein oxidation (32).

Primed Constant Isotope Infusions and Blood Sampling

Indwelling catheters (Insyte, Deseret Medicals, Sandy, UT) were inserted in an antecubital vein of each arm. One was used for the isotope infusion, the other for blood sampling. Following the collection of a baseline blood sample, primed constant infusions of [$^2H_5$] glycerol (Isotech Co., Miamisburg, OH) (prime: 1.5 μmol · kg^{-1}, infusion: 0.1 μmol · kg^{-1} · min^{-1}) (36,38), 1-^{13}C palmitate (infusion: 0.04 μmol · kg^{-1} · min^{-1}, in 5% albumin solution; no prime), 6,6-$^2H_2$ glucose (prime: 17.6 μmol · kg^{-1}; infusion: 0.22 μmol · kg^{-1} · min^{-1}; 99% enriched) were performed by a calibrated infusion pump (Harvard Apparatus, Natick, MA). Rates of infusion were determined by measuring the concentration of the infusate (duration of infusion was 180 min). Palmitate was bound to albumin, following previously described procedures (35). All solutions were prepared under sterile conditions and tested before use for sterility and pyrogenicity in an established independent laboratory, who found them all to be sterile and pyrogen-free.

Venous blood (3–7 ml) was drawn about 15 min prior to each test while subjects were sitting at a comfortable ambient temperature (22°C), as well as at min 60, 120, 135, 150, 165, and 180 in the climatic chamber. To ensure catheter patency without a heparin lock, we used a slow infusion of warm isotonic saline (~1 ml · min^{-1}) coupled with regular flushes of warm saline. The total volume of saline infused amounted to about 200 ml. A temperature controller maintained both the insulated saline bag and insulated i.v. line at 34–37°C. At the times specified above, blood samples were drawn into heparinized tubes chilled in an ice bath. For insulin analyses, Vacutainer® tubes containing EDTA were used. Plasma was promptly separated by centrifugation and kept frozen at −70°C until assayed for glycerol (2), free fatty acids (FFA; Wako Chemicals kit, Dallas, TX), palmitate (gas chromatography), glucose (YSI Glucose Analyzer, Yellow Springs, OH) and insulin (Pharmacia Diagnostics kit, Uppsala, Sweden). Plasma values were corrected for changes in plasma volume based on changes in hemoglobin (Sigma Chemicals kit, St. Louis, MO) and hematocrit, as before (28,33).

For analysis of 1-^{13}C palmitate, blood was collected and processed as described by Wolfe (38). After thawing the plasma sample, fatty acids were rapidly extracted from the plasma, separated by gas chromatography, derivatized to their methyl esters. The isotopic enrichment was determined by selected ion monitoring gas chromatography mass spectromery (GC MS). Blood for the analysis of [$^2H_5$] glycerol enrichment was collected and processed as before (38). An internal standard, 2-^{13}C glycerol, was added with the exception of the background sample, proteins were precipitated and the supernatant

was sequentially passed through ion exchange columns. Trimethylsilyl derivatives were formed and isotopic enrichment determined by GC MS using electron impact ionization. Blood for the analysis of 6,6-[$^2H_2$] glucose enrichment was collected and processed as before (38). Plasma proteins were precipitated and the supernatant passed sequentially through ion exchange columns. Pentaacetate derivatives were formed and isotopic enrichment determined by GC MS using the chemical ionization mode. All between and within assay coefficients of variation were less than 5%.

Calculations

The heat balance equation summarizes whole body heat exchanges in terms of heat production and heat loss (all variables expressed in $W \cdot m^{-2}$) (7,32,33) as follows:

$$S = M - (R + C) - E_{persp} - C_{resp} - E_{resp} \quad \text{Eq. 1}$$

where M is the metabolic rate, calculated from measured values of oxygen consumption and carbon dioxide production ($\dot{V}O_2$ and $\dot{V}CO_2$ in $L \cdot min^{-1}$ STPD; see below); R+C is the measured rate of dry heat exchange by radiation and convection, corrected for the thermal resistance of the transducer itself (32,33); E_{persp} is the calculated rate of evaporative heat loss from the skin (32,33); C_{resp} and E_{resp} are the calculated rates of convective and evaporative heat loss, respectively, by the respiratory tract (18); and S is the rate of heat storage, determined as the minute by minute balance of heat gains and heat losses. A negative value of S signifies a heat debt, a positive one, a heat gain. The total heat debt was then obtained by the summation of all rates of heat debt values and by converting $W \cdot m^{-2}$ to $kJ \cdot kg^{-1}$, knowing the subject's body surface area and body mass.

The rates of carbohydrate and lipid substrate oxidation (CHO_{ox}, and FAT_{ox} respectively, both in $g \cdot min^{-1}$) were calculated using the non-protein oxygen consumption (VO_{2np}) and the non-protein respiratory exchange ratio (NPRER) (11,34). Protein oxidation ($Prot_{ox}$) was assessed by analyzing the urinary urea nitrogen excretion (derived from the ~4 h urine collection and expressed as $g \cdot min^{-1}$) and correcting it for changes in the blood urea nitrogen, as previously described ($UREA_{corr}$; 11; Sigma Chemicals Co., Kit 640, St. Louis, MO). The calculations were performed as follows:

$$TERM = (NPRER - 0.707) \cdot 0.293^{-1} \quad \text{Eq. 2}$$

$$CHO_{ox} = TERM \cdot VO2_{np} \cdot 0.788^{-1} \quad \text{Eq. 3}$$

$$FAT_{ox} = (1 - TERM) \cdot VO2_{np} \cdot 2.0193^{-1} \quad \text{Eq. 4}$$

$$PROT_{ox} = UREA_{corr} \cdot 6.25 \quad \text{Eq. 5}$$

$$M = [(19.61 + TERM \cdot 1.51) \cdot VO2_{np} + (18.66 \cdot VO2_{prot})] \cdot 0.06^{-1} \cdot BSA^{-1} \quad \text{Eq. 6}$$

In Eq. 2–6, TERM represents the ratio of carbohydrate to non-protein oxidation; 0.293 is the difference between a NPRER of 1.000 and 0.707; $\dot{V}O_{2prot}$ is the $\dot{V}O_2$ due to protein oxidation, determined using 0.966 and 0.782 L of O_2 and CO_2 per g of protein; 0.788 is the average O_2 consumption required for the oxidation of 1 g of glycogen (0.829 $L \cdot g^{-1}$) or 1 g of glucose (0.744 $L \cdot g^{-1}$); 2.0193 corresponds to the O_2 consumption per gram of triglyceride ($L \cdot g^{-1}$); 6.25 is the conversion factor for urinary urea nitrogen to protein; 19.61 and 18.66 are the energy equivalents in $kJ \cdot L^{-1}$ oxygen when lipid and protein, respectively, are oxidized; 1.51 is the difference between the energy equivalent of 1 L O_2 at a NPRER of 1.000 and 0.707; 0.06 is a conversion factor to $J \cdot s^{-1}$ or W and finally, BSA is the body surface area in m^2 (11,34). The relative proportions of the average energy expenditure derived from carbohydrate (average of glucose and glycogen), lipids, and protein were determined using energy equivalents of 16.7, 39.3, and 18.0 $kJ \cdot g^{-1}$, respectively. The rates of fat or TG oxidation were converted from $g \cdot min^{-1}$ to its molar equivalent in $\mu mol \cdot kg^{-1} \cdot min^{-1}$, by assuming that palmityl-oleyl TG (860 $g \cdot mol^{-1}$) represented a typical TG.

After having obtained relative steady-state of isotopic enrichments at least during the last hour of experimentation (i.e., between min 120 and 180), the rates of appearance (R_a) of plasma glycerol, palmitate and glucose (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were calculated during this time period using steady-state kinetics modified for use with stable isotopes (5,38):

$$R_a = [(IE_{Inf} \cdot IE^{-1}_{Plasma}) - 1] \cdot F \quad \text{Eq. 7}$$

where IE_{Inf} and IE_{Plasma} represent the isotopic enrichment (in mol %) of the infusate and plasma samples respectively, and F is the infusion rate in $\mu mol \cdot kg^{-1} \cdot min^{-1}$. FFA R_a was calculated by dividing palmitate R_a by the percentage of contribution of palmitate to total FFA concentration. Under steady-state conditions, the rate of disappearance (R_d) was assumed to be equal to R_a (5,16,19,38). All stable isotope-related calculations pertain to the relative steady-state conditions of the last hour of exposure.

Lipolysis was calculated as three times the glycerol R_a. The rationale for this calculation is that for each glycerol released from TG, three fatty acids must also be released. Thus, if fatty acids appear in the plasma at a rate less than three times the rate of glycerol R_a, then some reesterification must have occurred (38). Reesterification takes place in two forms (21,36–38). Extracellular (EC) TG/FFA recycling can be calculated as the difference between FFA R_a and FAT_{ox} (21,36–38). Intracellular (white adipose tissue) TG/FFA recycling was assessed as the difference between the rate of lipolysis and the FFA R_a (38). Thus, total recycling represents the sum of the above two rates of recycling or the difference between lipolysis and FAT_{ox} (12). With the available CHO metabolism data, it was then possible to estimate the plasma vs. intramuscular contribution of CHO oxidative metabolism via the following equation. Knowing that plasma $R_a = R_{ox} + R_{nonox}$ (14,25), it is possible to rearrange the equation by substituting R_{ox} (an unknown) for its equivalent so that:

$$\text{Glucose } R_a = (CHO_{ox} - \text{Glycogen Muscle}_{ox}) + R_{nonox} \quad \text{Eq. 8}$$

where R_{ox} is the rate of plasma substrate (glucose) oxidation; R_{nonox} is the rate of plasma substrate non-oxidative metabolism and Muscle $_{ox}$ is the rate of intramuscular substrate (glycogen) oxidation (38). For CHO, assuming

TABLE I. SUMMARY OF KEY THERMAL DATA.

	Thermal Neutrality	Cold
T_{re} (0 min) °C	36.98 ± 0.10	36.90 ± 0.13
T_{re} (180 min) °C	36.72 ± 0.06	36.08 ± 0.23*
delta T_{re}	−0.26 ± 0.09	−0.82 ± 0.21*
T_{sk} (0 min) °C	32.5 ± 0.19	31.30 ± 0.19
T_{sk} (180 min) °C	33.8 ± 0.12	22.18 ± 0.47**
delta T_{sk}	1.3 ± 0.10	−9.1 ± 0.21**
Final S (180 min) kJ · kg^{-1}	0.4 ± 0.2	−22.8 ± 1.8**

* $p < 0.05$.
** $p < 0.01$.

that plasma glucose R_a is completely oxidized (5,21), thus equivalent to maximum rates of plasma glucose oxidation, one can estimate the minimum rates of glycogen oxidation (and lactate) (3,8,21,34). Note that in Eq. 8, the minimum $Muscle_{ox}$ estimate remains valid and would only be higher than the minimum if R_{nonox} would be greater than zero. For FFA, an equivalent Eq. 8 could similarly be applied to solve TG $Muscle_{ox}$ [FFA R_a = (FFA_{ox} − TG $Muscle_{ox}$) + R_{nonox}] since all other terms are known. However, it was not used since this commonly used determination of R_{nonox} (R_{nonox} = FFA R_a − FAT_{ox}) (see the above EC recycling; 21, 36–38) already assumes minimal rates of TG $Muscle_{ox}$ to yield maximal rates of nonoxidative disposal of fatty acids. As such, the above calculations are necessarily approximate.

Statistics

The main effects of time and treatments (thermoneutral vs. cold) as well as time/treatment interactions were tested by repeated measures analyses of variance (ANOVA) on all thermal and metabolic data (6). Paired *t*-tests, adjusted for multiple comparisons when required (6), were used to identify the location of significant differences (at least $p < 0.05$) when the ANOVA yielded a significant F-ratio for the interaction. Results are expressed as mean ± SEM.

RESULTS

As expected, 3 h of cold exposure reduced T_{sk} by more than 9°C whereas the drop in T_{re} was almost 1°C compared with the trial at thermal neutrality ($p < 0.05$; Table I). The rates of heat loss markedly increased in the cold, particularly dry heat losses (R+C; final values at min 180 at thermoneutrality vs. cold tests: 33 ± 1 vs. 149 ± 5 W · m^{-2} respectively, $p < 0.01$). Although final wet (E_{persp}; at min 180: 2.0 ± 0.2 vs 6.1 ± 0.8 W · m^{-2}, $p < 0.01$) and respiratory heat losses (at min 180: C_{resp}: 0.2 ± 0.0 vs. 3.9 ± 0.5 W · m^{-2}, $p < 0.01$; E_{resp}: 3.0 ± 0.1 vs. 10.0 ± 0.9 W · m^{-2}, $p < 0.01$) were also increased by the cold, quantitatively they were much less important. Overall, it is therefore not surprising that the cold test produced a large cumulative heat debt whereas a very small heat gain was observed at thermal neutrality ($p < 0.01$, Table I).

M was 38.9 ± 1.3 W · m^{-2} at rest before entering the climatic suite. At thermal neutrality, there was no significant change in M during the 3 h exposure (Fig. 1). M was significantly affected by a main effect of cold, however, increasing gradually to 2.7 times resting values at min 120, where it stayed relatively stable, at about 110 W · m^{-2} ($p < 0.01$; Fig. 1). In part, the cold-induced increase in M was due to an increase in whole body CHO

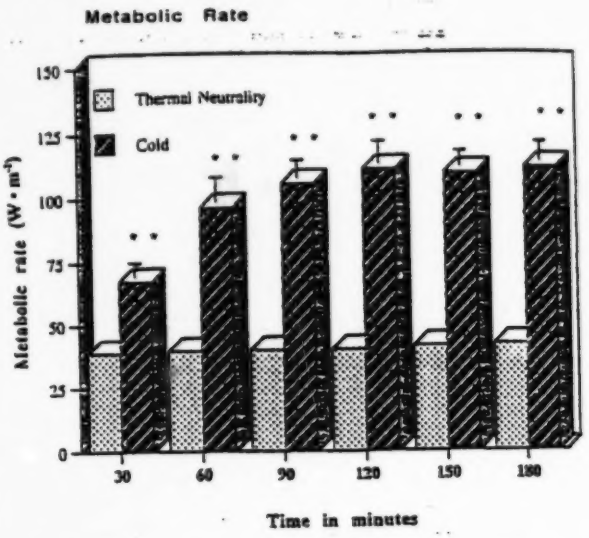

Fig. 1. Metabolic rate (M, in W · m^{-2}) during a 3 h exposure to either thermal neutrality (29°C) or cold (5°C). Fasting subjects were sitting at rest in the climatic chamber wearing only jogging shorts. Results were calculated from $\dot{V}O_2$ and NPRER as in Methods and each data point represents the mean of the previous 30 min (i.e., data at 30 min represent min 1–30). Repeated measures ANOVA indicated that M was affected by a significant main effect of cold ($p < 0.01$). The symbols * or ** indicate a significant difference between tests at $p < 0.05$ or $p < 0.01$, respectively. Results are mean ± SEM throughout.

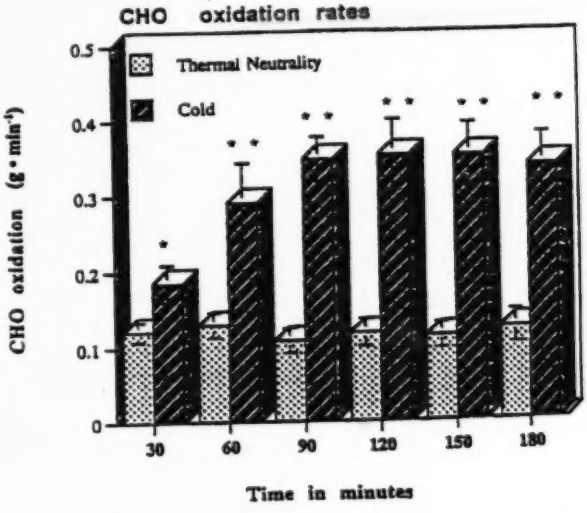

Fig. 2. Rates of carbohydrate oxidation (CHO_{ox}) during the 3 h test at thermal neutrality or in the cold. Symbols for statistical differences are as in Fig. 1.

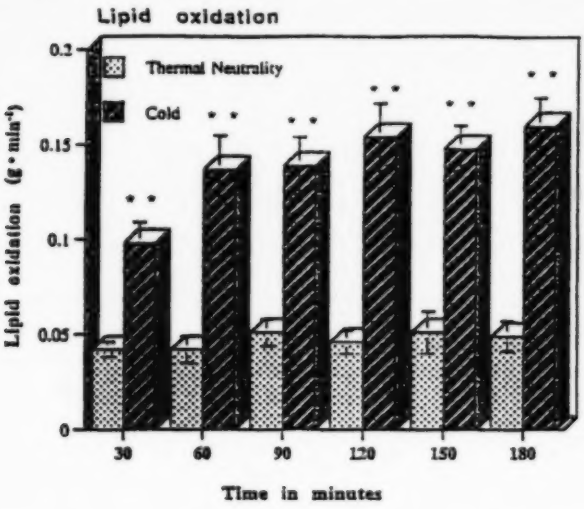

Fig. 3. Rates of fat oxidation (FAT_{ox}) during the 3 h test at thermal neutrality or in the cold. Symbols for statistical differences are as in Fig. 1.

oxidation (Fig. 2). The rates of CHO_{ox} were higher in the cold than at thermal neutrality at all points in time ($p < 0.05$); overall the 3-h average in CHO_{ox} was about 2-fold greater in the cold ($p < 0.01$). As with CHO_{ox}, FAT_{ox} was also affected by a significant interaction of effects. Fig. 3 shows that the rates of FAT_{ox} in the cold were significantly greater at all points in time ($p < 0.05$). Over the 3 h period, cold increased FAT_{ox} by more than two fold ($p < 0.01$).

Fig. 4A describes the plasma isotopic enrichment of $^2H_2$ glucose as a function of time. In both physiological conditions, relative steady-states were obtained in the last hour of testing. The enrichment profile, from min 120 to 180, was reduced by a significant main effect of cold exposure. Fig. 4B summarizes the measured and calculated values related to the steady-state kinetics of the $^2H_2$-glucose as well as rates of steady-state whole body CHO_{ox}. Cold exposure increased CHO_{ox} by 195%, compared with thermal neutrality ($p < 0.05$) whereas it increased the plasma glucose R_a by 45% ($p < 0.05$). Assuming that R_a is completely oxidized in the cold (equivalent to maximum rates of plasma glucose oxidation), the minimum rates of glycogen (and lactate) oxidation would be the difference between CHO_{ox} and glucose R_a, about 12.0 ± 1.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Under the present laboratory conditions, the oxidation of plasma glucose in the cold would thus account for a maximum of about 52% of the whole body CHO_{ox}, whereas a minimum of 48% could be attributed to the combination of glycogen (and lactate) oxidation. Overall, cold thermogenesis was derived from FAT_{ox} and CHO_{ox} according to quite comparable proportions as above: 48% and 46%, respectively, of the average energy expenditure, whereas the rest of the heat production was attributed to protein oxidation (6%).

Fig. 5 summarizes the effect of cold exposure on plasma levels of glycerol (Fig. 5A), FFA (Fig. 5B) and the isotopic enrichments of $^2H_5$-glycerol (Fig. 5C). The 3 h cold stress did significantly increase overall levels of plasma glycerol (222%) and FFA (107%) whereas relative steady state isotopic enrichment was reduced by about half ($p < 0.05$). Correspondingly, cold exposure enhanced the $^2H_5$-glycerol R_a by 184% (Fig. 6A; $p < 0.05$). Lipolysis being three times glycerol R_a, was thus increased by exactly the same amount. FFA R_a was enhanced by 220% in the cold, up to 14.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.05$; Fig. 6B). A relatively similar 194% increment was found with FAT_{ox}. With FFA R_a remaining at more than twice greater rates than FAT_{ox}, which only increased up to 6.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.05$), clearly both oxidative disposal and non-oxidative disposal (or TG/FFA cycling) were involved to account for all FFA disposal. Fig. 7 indeed clearly shows that the total recycling was increased by 197% in the cold, primarily due to extracellular recycling ($p < 0.05$) and not intracellular recycling. Plasma insulin levels were not significantly affected by the treatment (min 0 vs. 180 at neutrality: 7.9 ± 1.3 vs.

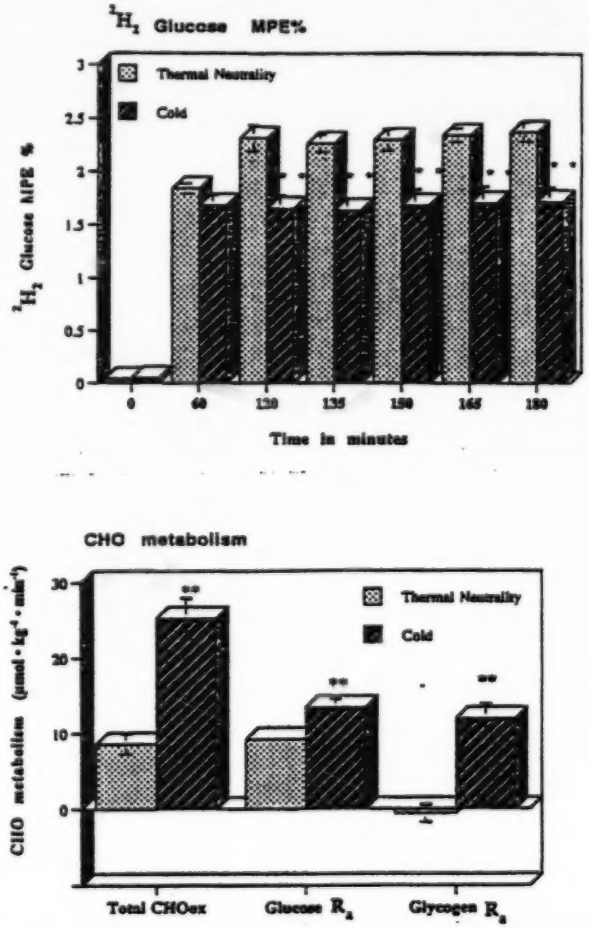

Fig. 4. Plasma $^2H_2$-glucose enrichment (mole per cent excess, MPE%; Fig. 4A, top) as a function of time during the test at thermal neutrality or in the cold. Fig. 4B (bottom) describes: 1) the rates of steady-state whole body carbohydrate oxidation (total CHO_{ox}); 2) the rates of plasma glucose appearance (or disappearance) (Glucose R_a); and 3) the calculated rates of glycogen utilization (Glycogen R_a). Symbols for statistical differences are as in Fig. 1.

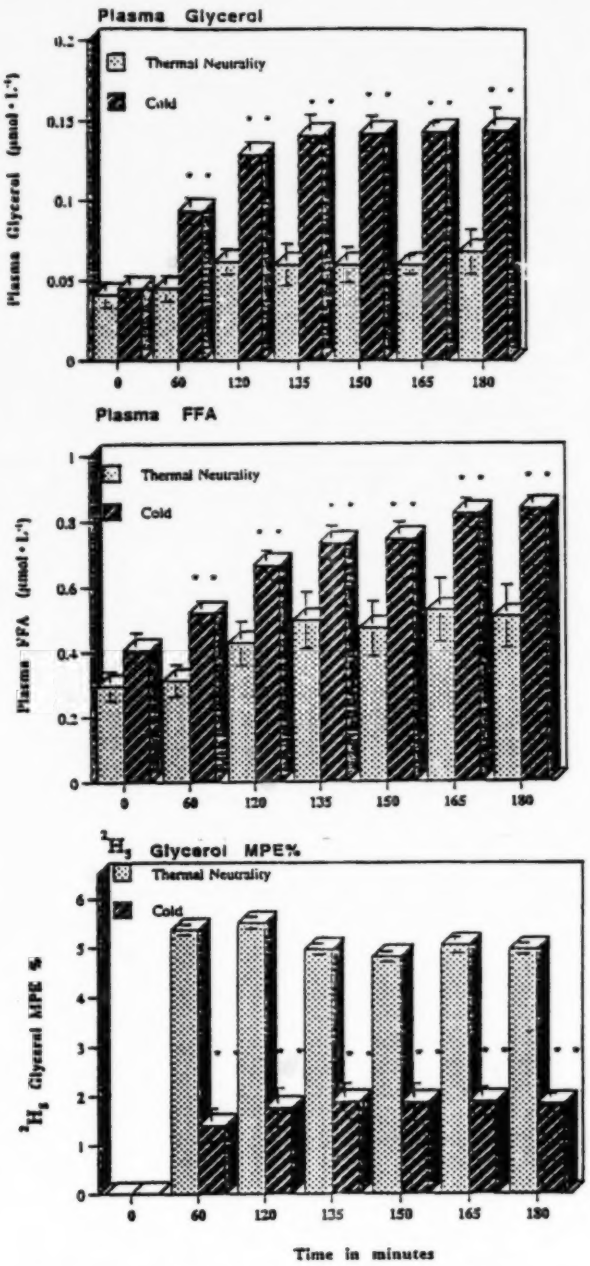

Fig. 5. Plasma levels of glycerol (Fig. 5A, top), free fatty acids (mmol·L^{-1}; Fig. 5B, middle) and $^2H_2$-Glycerol enrichment (mole per cent excess, MPE%; Fig. 5C, bottom) as a function of time during the test at thermal neutrality or in the cold. Symbols for statistical differences are as in Fig. 1.

6.1 ± 1.0 μU·ml^{-1}, respectively; min 0 vs. 180 in the cold: 7.3 ± 1.3 vs. 5.4 ± 1.0 μU·ml^{-1}, respectively).

DISCUSSION

The major new findings of this study are that cold exposure not only shifts lipid kinetics toward an increased mobilization turnover as well as oxidative disposal of fatty acids, but it also shifts it toward an enhanced non-oxidative disposal of fatty acids.

Indeed results showed that cold increases lipolysis, FFA R_a, FAT_{ox}, as well as extracellular TG/FFA cycling (Figs. 3, 6A, 6B, 7). The observation that FFA R_a largely exceeded FAT_{ox} (Fig. 6B) confirms our hypothesis and clearly indicates a dissociation, or uncoupling, between the availability and the oxidation of fat. This phenomenon carries several implications for long term exposure to physiological conditions of enhanced energy expenditure such as cold stress. Before reviewing these implications, it would appear advantageous to review the various model assumptions employed in the present study and to demonstrate the validity of our own absolute values of lipid kinetics.

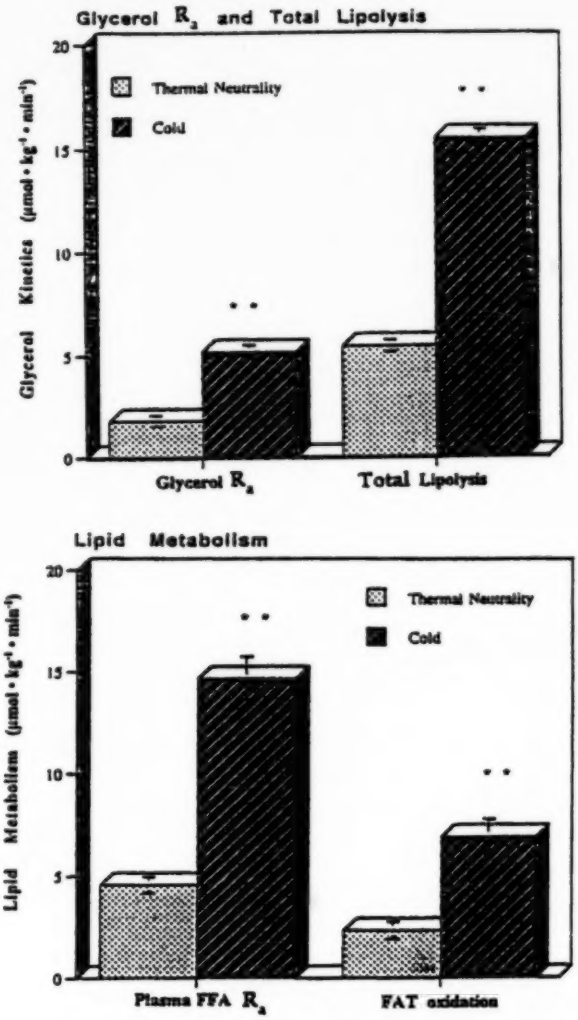

Fig. 6. Rates of plasma glycerol R_a and total lipolysis (Fig. 6A, top) during the test at thermal neutrality or in the cold. Fig. 6B (bottom) describes the rates of plasma FFA R_a and whole body lipid oxidation during the test at thermal neutrality or in the cold. Symbols for statistical differences are as in Fig. 1.

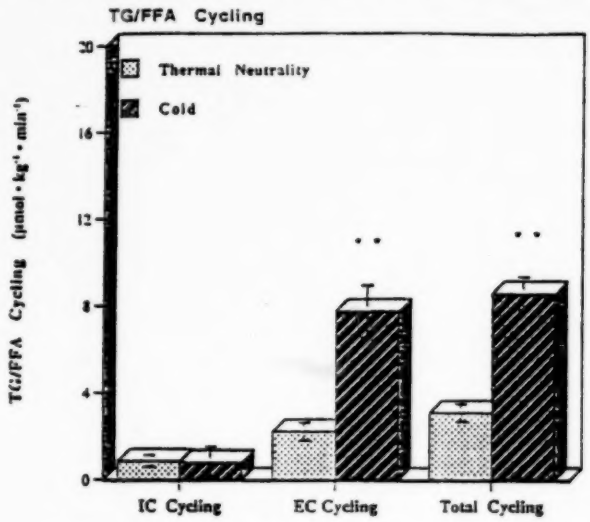

Fig. 7. Rates of intracellular (IC) recycling, extracellular (EC) recycling and total recycling during the test at thermal neutrality or in the cold. Symbols for statistical differences are as in Fig. 1.

Our absolute values of glycerol R_a and palmitate R_a are entirely comparable to other published reports. At thermal neutrality, we have reported values of 1.8 and 4.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for glycerol and palmitate R_a, respectively, which represent values quite similar to 2 and 5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ published elsewhere (12,15). Further, other studies have also shown that cold exposure, particularly in animals, enhanced plasma levels of FFA, glycerol and catecholamines as well as lipolysis and free fatty acid turnover (for a review see 10; see Figs. 5A, 5B, 6A, 6B). However, none of these studies had previously compared oxidative to non-oxidative FFA disposal in the cold.

Model Assumptions

In the present study, we assumed that glycerol R_a represents a reliable index of lipolysis. There seems to be ample evidence to support this assumption. White adipose tissue has no glycerol kinase so all glycerol released will appear in plasma (20,37). Further, glycerol is not produced metabolically by any process other than lipolysis and an underestimation of lipolysis due to partial hydrolysis of TG is very unlikely (37). Finally, it is also established that in contrast to glycerol R_a, plasma FFA or glycerol levels or even FFA R_a remain unreliable as indices of lipolysis, particularly during physiological conditions of enhanced energy expenditure (12).

Another assumption is that FFA R_a is an accurate measure of FFA turnover. It would be possible to underestimate FFA R_a if fatty acids released by lipolysis would be directly oxidized by adjacent tissues without entering the plasma. However, there is evidence that there is little oxidation of fatty acids released by lipolysis, that do not enter the plasma (37). Further, ^{13}C-palmitate represents one of the most widely used tracers of FFA R_a in humans (38). It is also assumed that FAT_{ox} derived from indirect calorimetry reliably reflects the whole body rates of fat oxidation. There is abundant evidence to document the reliability of the calculations and this topic has been reviewed in details on numerous occasions (11). Further, FAT_{ox} is also considered to result from the oxidation of fatty acids derived from white adipose tissue and intramuscular stores, knowing that intravascular hydrolysis of TG is negligible on several accounts. First, we already know that in the cold, TG levels as well as the clearance of plasma TG, remain unchanged in humans (31). Second, it is also established that when using a fasting protocol (see Methods), plasma TG metabolism is not very active in the first place (21). Finally, uptake of plasma-derived TG is small and occurs very slowly, even during prolonged exercise (15). Thus, plasma FFA and intramuscular TG are likely to be the only major sources of fatty acids for oxidation, particularly in fasting individuals. Further studies are nevertheless needed to confirm this concept. Changes in intramuscular TG are also difficult to estimate, particularly without any direct measurements of ^{13}C-derived rates of plasma FFA oxidation. The commonly used method of assessing R_{nonox} (38) yields maximal rates of R_{nonox} by assuming minimal rates of TG $Muscle_{ox}$. Another technique that has been used to assess TG $Muscle_{ox}$ is to substract FFA R_a from FAT_{ox} (20). However, this can only be used when FAT_{ox} (oxidation) exceeds the plasma availability (20), an impossibility in the present study (see Fig. 6).

Fate of Fatty Acids

The uncoupling between the availability and the oxidation of fatty acids carries several implications. The primary one is that clearly there are factors other than the availability of fatty acids that are regulating fatty acid oxidation (1). The increased cycling in the cold could allow for an increase FAT_{ox} with no increment in lipolysis since additional fatty acids are being released and then re-esterified without oxidation. Fat availability is therefore not limiting as cycling primes for more oxidation. Factors such as enzymatic capacity to transport and to oxidize fat in the mitochondria are likely candidates (20,22). Indeed, recent data suggest that glucose and/or insulin regulate FAT_{ox} by controlling the rate of long-chain fatty acid entrance into the mitochondria (23).

It is interesting to note that similar physiological situations where FFA R_a (or availability) exceeded FAT_{ox} (or oxidation), by an amount corresponding to nonoxidative disposal of fatty acids, have been observed before (14,25). Further, relatively similar absolute levels of TG/FFA cycling have been reported in control subjects (3.5 ± 0.4 vs. 3.1 ± 0.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the present study), although absolute levels reported in burn patients were clearly higher that in the present cold-exposed subjects (15.8 ± 1.3 vs. 8.6 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively) (36). Knowing the relative similarities between the hypermetabolic state of burn patients and that of cold-exposed subjects, their larger increase in TG/FFA cycle could be attributed to a greater enhancement in β-adrenergic stimulation (36). There are many other factors which are known to affect recycling. Within white adipose tissue, the primary factors could well be the ability to carry away fatty acids (via blood flow or availability of albumin binding sites) and the availability of glucose

to produce glycerol 3-phosphate for reesterification. Since intracellular cycling rates remained unchanged (Fig. 6), these two latter factors do not seem to be affected by the cold. While it is not clear by which mechanisms cold increased extracellular cycling, it is possible that it occurred through a combination of increased plasma FFA levels, an unchanged liver blood flow, and an increased stimulation of FAT_{ox} (37), although it still remains to be demonstrated. It should also be noted that cycling requires energy and produces heat, but does not result in the net flux of products. Fatty acids released by lipolysis are reesterified rather than oxidized and the energy cost associated with this cycling can be easily calculated since it is known that eight ATP are utilized for each mol of TG cycled (17); TG/FFA cycling thus amounts to about 3% of M at rest as well as 3% in the cold.

In the present study, FAT_{ox} was mainly dependent on white adipose tissue-derived fatty acids and a negligible amount to intramuscular TG. Further, the assessment of *maximal* rates of nonoxidative disposal are tied to *minimal* rates of TG $Muscle_{ox}$, an assumption in line with the estimation of small rates of TG $Muscle_{ox}$ even when FAT_{ox} exceeded FFA R_a, clearly the reverse of the present of the present study (20). In general, this is comparable to data in cold-exposed dogs where adipose tissue-derived FFA represented a contribution to M as high as 64% of M (19). In the present study, it was estimated that FFA could contribute to about 48% of M. This is quite different from the observations with exercise regimens where intramuscular TG hydrolysis represented a significant fraction of FAT_{ox}. Indeed, it has been reported that in such cases, FAT_{ox} becomes equal or exceeds FFA R_a (the reverse of Fig. 6 and the best example of intramuscular TG hydrolysis) since fatty acids would be oxidized in muscle cells without ever appearing in the plasma (15). Further, it is only at relatively high exercise intensities that intramuscular lipolysis becomes stimulated (38), particularly in trained athletes (22). In several studies (see 15), though not all (24), exercise in trained athletes produced a shift to non-plasma or intramuscular source of fatty acids for oxidation because of decrements in FFA levels, in FFA R_a, in FFA R_{ox} and in lipolysis due to decrease in adrenal activity (15). Humans and rats, in contrast to migratory birds, do not accumulate large amounts of TG in their muscles. Further, usable TG deposits appear to be depleted within 2–3 h of continuous heavy exertion (9). We therefore suspect that since cold stress is not performed at a similarly high level of exertion, a much longer continuous exposure to cold for ~8–9 h may be necessary to observe an increase in muscle TG oxidation, although this needs to be confirmed.

Fate of Carbohydrates

The results of the present study confirm that cold exposure in humans enhances the rate of disappearance of plasma glucose (Fig. 5). This observation corroborates in humans previous animal experiments which documented similar cold-induced increases in R_a (16), cold-induced improvements in glucose and skeletal muscle glucose uptake (26,27) as well as other human studies describing increases in whole body carbohydrate oxidation and glucose tolerance with cold stress (28,29). It should also be noted that the present absolute glucose R_a values at rest were similar to other published values (9 vs. 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in 13) and the cold-induced increase of 46% in R_a is comparable in magnitude, although slightly higher than a previous lesser stressful cold test (34). Though the fate of the increased disappearance of plasma glucose is not clear, assuming that R_a is completely oxidized in the cold, one can estimate the minimum oxidation rate of glycogen (and lactate) (3,8,21), which would amount to about 12.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, or about 48% of CHO_{ox} (see Results). Such an estimate is difficult to measure in humans since it depends on two elusive factors: the average rate of glycogen utilization in cold-exposed humans, and the active shivering muscle mass utilizing glycogen at that rate. To obtain this estimate without a series of biopsies, we assumed that R_{nonox} was negligible. What would happen if this were not the case? The minimum $Muscle_{ox}$ estimate of Eq. 8 would remain valid and would only be higher if R_{nonox} would be greater than zero. We would thus overestimate the (maximum) rate of plasma oxidation and underestimate the (minimum) rate of glycogen oxidation. Further investigation is nevertheless required.

In summary, even though lipolysis and FFA R_a are greatly increased by cold stress in humans, the present results demonstrate that only about half the rate of FFA R_a is ultimately oxidized. This demonstrates a dissociation or uncoupling with fatty acids availability being more than two-fold greater than FAT_{ox}. It also suggests that under the present cold conditions in humans: 1) nonoxidative FFA disposal or TG/FFA cycling is significant; 2) white adipose tissue-derived fatty acids could easily account for most of the whole body fat oxidation; and 3) further research is needed to clarify the contribution of intramuscular TG. It is also worthwhile to note that the results further emphasize the importance of the TG/FFA cycle in amplifying the ability of stored TG to react quickly to major changes in M such as those induced by a sustained cold exposure, without the need to further enhance lipolysis.

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REFERENCES

1. Boden G, Chen X, Desantis RA, Kendrick Z. Effects of insulin on fatty acid reesterification in healthy subjects. Diabetes 1993; 42:1588–93.
2. Boobis LH, Maughan RJ. A simple one step enzymatic fluorometric method for the determination of glycerol in 20 μl of plasma. Clin Chim Acta 1983; 132:173–9.
3. Bosch AN, Dennis SC, Noakes TD. Influence of carbohydrate loading on fuel substrate turnover and oxidation during prolonged exercise. J Appl Physiol 1993; 74:1921–7.
4. Burton AC, Edholm OG. Man in a cold environment. New York: Hafner Publishers, 1955.

5. Coggan AR, Kohrt WR, Spina RJ, et al. Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men. J Appl Physiol 1990; 68:990–6.
6. Glantz SA. Primer of biostatistics. New York: McGraw-Hill, 1981; 260–1.
7. Hardy JD, Stolwijk JAJ. Partitional calorimetry studies of man during exposures to thermal transients. J Appl Physiol 1966; 21:1799–1806.
8. Hawley JA, Bosch AN, Weltan SM, et al. Glucose kinetics during prolonged exercise in euglycaemic and hyperglycaemic subjects. Pflug Archiv 1994; 426(5):378–386.
9. Holloszy JO. Utilization of fatty acids during exercise. In: Taylor AW, Gollnick PD, Green HJ, et al., ed. Biochemistry of exercise VII. Vol. 21. Champaign IL: University Press, 1990:319–27.
10. Jacobs I, Martineau L, Vallerand AL. Thermoregulatory thermogenesis in humans during cold stress. In: Holloszy JO, ed. Exercise and Sport Sciences Reviews, Vol. 22. Baltimore: Williams & Wilkins, 1994; 221–50.
11. Jéquier E, AchesonK, Schutz KY. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987; 7:187–208.
12. Klein S, Coyle EF, Wolfe RR. Fat metabolism during low-intensity exercise in endurance-trained and untrained men. Am J Physiol 1994; 267:E934–E940.
13. Knapik JJ, Meredith CN, Jones BH, et al. Influence of fasting on carbohydrate and fat metabolism during rest and exercise in men. J Appl Physiol 1988; 64:1923–9.
14. Mansell PI, Macdonald IA. The effect of starvation on insulin-induced glucose disposal and thermogenesis in humans. Metabolism 1990; 39(5):502–10.
15. Martin WH III, Dalsky GP, Hurley BF, et al. Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. Am J Physiol 1993; 265:E708–E714.
16. Minaire Y, Forichon J, Jomain J, Dallevet G. Independence of circulating insulin levels of the increased glucose turnover in shivering dogs. Experientia 1981; 37:745–7.
17. Newsholme EA, Crabtree B. Substrate cycles in metabolic regulation and in heat generation. Biochem Soc Symp 1992; 41:61–109.
18. Nishi Y. Measurement of thermal balance of man. In: Cena K, Clark JA, ed. Bioengineering, thermal physiology and comfort. New York: Elsevier Publ., 1981: 29–39.
19. Pernod A, Vincent-Falquet JC, Jomain M-J, Minaire Y. Glucose and glycogen as fuels for thermogenesis in cold-exposed dogs. Life Sci 1972; 11(I):475–82.
20. Phillips SM, Green HJ, Tarnopolsky MA, et al. Effects of training duration of substrate turnover and oxidation during exercise. J Appl Physiol 1996; 81(5):2182–91.
21. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol 1993; 265:E380–E391.
22. Romijn JA, Coyle EF, Sidossis LS, et al. Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. J Appl Physiol 1995; 79(6):1939–45.
23. Sidossis LS, Stuart CA, Shulman GI, et al. Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. J Clin Invest 1996; 98:2244–50.
24. Starling RD, Trappe TA, Parcell AC, et al. Effects of diet on muscle triglyceride and endurance performance. J Appl Physiol 1997; 82(4):1185–9.
25. Toth MJ, Arciero PJ, Gardner AW, et al. Rates of free fatty acid appearance and fat oxidation in healthy younger and older men. J Appl Physiol 1996; 80(2):506–11.
26. Vallerand AL, Lupien J, Bukowiecki LJ. Interactions of cold exposure and starvation on glucose tolerance and insulin response. Am J Physiol 1983; 245:E575–E581.
27. Vallerand AL, Pérusse F, Bukowiecki LJ. Cold exposure potentiates the effect of insulin on *in vivo* glucose uptake. Am J Physiol 1987; 253:E179–E186.
28. Vallerand AL, Frim J, Kavanagh MF. Plasma glucose and insulin responses to oral and intravenous glucose in cold-exposed humans. J Appl Physiol 1988; 65:2395–9.
29. Vallerand AL, Jacobs I. Rates of energy substrate utilization during human cold exposure. Eur J Appl Physiol 1989; 58:873–8.
30. Vallerand AL, Limmer R., Schmegner IF. Computer acquisition and analysis of skin temperature and heat flow data from heat flux transducers. Computer Methodsods Programs Biomedicine 1989; 30:279–82.
31. Vallerand AL, Jacobs I. Influence of cold exposure on plasma triglyceride clearance in humans. Metabolism 1990; 39(11):1211–8.
32. Vallerand AL, Tikuisis P, Ducharme MB, Jacobs I. Is energy substrate mobilization a limiting factor for cold thermogenesis? Eur J Appl Physiol 1993; 67:239–44.
33. Vallerand AL, Jacobs I. Effects of a high-energy food supplement on cold-induced thermogenesis. North York, Ont.: Defence & Civil Inst Environ Med. 1993: 1–27; Report # 93–36.
34. Vallerand AL, Zamecnik J, Jacobs I. Plasma glucose turnover during cold stress in humans. J Appl Physiol 1995; 78(4):1296–302.
35. Wolfe RR, Evans JE, Mullany CJ, Burke JF. Measurement of plasma free fatty acid turnover and oxidation using [1-^{13}C] palmitic acid. Biomed Mass Spectrom 1980; 7:168–71.
36. Wolfe RR, Herndon DN, Jahoor F, et al. Effect of severe burn injury on substrate cycling by glucose and fatty acids. N Engl J Med 1987; 317:403–8.
37. Wolfe RR, Klein S, Carraro F, Weber J-M. Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. Am J Physiol 1990; 258:E382–E389.
38. Wolfe RR. Radioactive and stable isotope tracers in biomedicine. Principles and practice of kinetic analysis. New York: Wiley-Liss, 1992.